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New Distributional Records of Onchidiid Slugs from Coringa Wildlife Sanctuary, Andhra Pradesh

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Abstract

Coringa Wildlife Sanctuary is one of unique ecosystems in the State of Andhra Pradesh which is endowed with a complex of riverine, estuarine, mangrove, coastal and marine ecosystems with an area cover of 235.70 sq. km. During the faunal surveys at the sanctuary, two species of onchidiid slugs *viz*., *Onchidium typhae* Buchannan, 1800 and *Platevindex tigrinus* (Stoliczka, 1869) were identified as a new distributional record to this region. *Onchidium typhae* which had morphological variation among individuals were confirmed using molecular analysis.

Keywords: Mangrove, Mollusca, *Onchidium*, *Platevindex*, Slug

Introduction

Onchidiids are true slugs without internal shell. Most of the species are marine and live in the upper intertidal zone, in rocky, sandy, or muddy habitats, including mangroves (Dayrat, 2009). While two species *viz*., *Onchidium typhae* Buchannan, 1800, and *O. stuxbergi* (Westerlund, 1883) live in brackish habitats and tolerate freshwater. The members of the family Onchidiidae feed on organic detritus and unicellular algae in the surface mud (Sun *et al*., 2014) and helping in the nutrient recycling. Also, some species like *Peronia verruculata* (Cuvier, 1830) is also used as food in some part of the world (Solanki *et al*., 2017).

The systematics of the Onchidiidae, one of the higher clades of pulmonate gastropods, has been difficult for decades (Dayrat *et al*., 2016). As per literature studies, altogether 143 species of onchidiids under 19 genera are reported across the globe (Dayrat, 2009). Until recently, the number of onchidiids reported from India was very less accounting for only six species (Dey, 2016). Currently, 16 species of onchidiid slugs are reported from various parts of India (Dey, 2016; Dayrat *et al*., 2016; 2017; 2019a; 2019b; 2020; Goulding *et al*., 2018a; 2018b).

The first onchidiid slug recorded from India was *Onchidium typhae* with its type locality at Port Canning, West Bengal (currently Canning, South 24 Parganas, West Bengal) (Buchannan, 1800). During 1869, three species of onchidiid slugs were described from the same region by Stoliczka. Thereafter, there were no major works on these slugs from the Indian region. Recently, Benoit Dayrat of University of California/Pennsylvania State University had undertaken a study for revising the taxonomy of the entire family, using an integrative approach (Dayrat, 2005), the collection localities of that included some of the major mangrove regions of India also. These studies have resulted in some new species and regional records from India (Dayrat *et al*., 2016, 2017, 2019a; 2019b; 2020; Goulding *et al*., 2018a; 2018b).

Material and Methods

The current study was carried out during the exploration of "Fauna of Coringa wildlife Sanctuary, Andhra Pradesh" during December 2018 and November 2019. Coringa Wildlife Sanctuary is one of the 21 wildlife sanctuaries in Andhra Pradesh which is endowed with a complex of riverine, estuarine, mangrove, coastal and marine ecosystems with an area cover of 235.70 sq. km.

Collection and Preservation

The collections were made by manual search method and handpicking. The collected animals were asphyxiated

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with freshwater. The completely relaxed and freshly dead animals were fixed in 10% formalin (in seawater) solution and later preserved in 70% ethanol for long term preservation. The animals/body parts for the DNA analysis were directly preserved in 100% ethanol. The preserved animals are deposited in the National Zoological Collections of the Zoological Survey of India. The animals were identified using Dayrat *et al.* (2016) and Dey (2006). The length of the animal mentioned in the work is the maximum length of the crawling animal measured in the field.

Molecular Analysis

Total genomic DNA extraction was performed using DNeasy Blood and Tissue Kit (QIAGEN) according to the manufacturer's protocol. The target 500-bp fragment of 16s was amplified by Polymerase Chain Reaction (PCR) using universal primer pair, 16s gene were 16SF 5'CGCCTGTTTATCAAAAACAT3',16SR 3'CCGGTCTGAACTCAGATCATGT5'. Thermal cycle parameters for 25µl initial step of 3min at 95°C followed by 35 cycles of 30s at 94°C, 30s at 47°C and 40s at 72° C followed in turn by final extension of 10 min at 72°C. The PCR products were tested by electrophoresis on an agar gel and, if a single band was observed, were purified using a QI Aquick PCR purification kit (QIAGEN Inc). PCR products were directly sequenced in both directions by the automated sequencer (ABI Prism 3730 XL DNA Analyzer). Resulting chromatograms were evaluated for miscalls and ambiguities and assembled into contigs in Chromas 2.6.4. The sequences were individually checked, verified for protein coding frame-shifts to avoid pseudogenes. Consensus files were aligned. Sequences were checked in Basic Local Alignment Search Tool (BLAST) to find the closest sequences available in the GenBank (www.ncbi.nlm.nih.gov). Sequence generated as part of the study is deposited in GenBank.

Results

A total of 15 samples belonging to two genera and two species are identified. The details of the identified slugs are given below

Taxonomic Account

Phylum MOLLUSCA Linnaeus, 1758
Class GASTROPODA Cuvier, 1795
Subclass HETEROBRANCHIA Burmeister, 1837
Infraclass EUTHYNEURA Spengel, 1881
Subterclass TECTIPLEURA Schrödl, Jörger, Klussmann-Kolb & Wilson, 2011
Superorder EUPULMONATA Haszprunar & Huber, 1990
Order SYSTELLOMATOPHORA Pilsbry, 1948
Family ONCHIDIIDAE Rafinesque, 1815
Genus ***Onchidium*** Buchannan, 1800
Onchidium typhae Buchannan, 1800

Material examined: M33049/9 (Mollusca Section, ZSI, Kolkata), 2 exs, Length: 4.5 cm & 6 cm, 19.12.2018, Coll: Sreeraj CR, Chollangi, Coringa Wildlife Sanctuary, East Godavari, Andhra Pradesh. ZSI/SbRC/KN3088 (Sunderban Regional Centre, ZSI)10 exs, Length: 5.3±1.6 cm, 29.11.2019, Coll: Sreeraj CR, Chollangi, Coringa Wildlife Sanctuary, East Godavari, Andhra Pradesh.

GenBank Accession Numbers: MW459188; 450 bp; large subunit ribosomal RNA gene, partial sequence; mitochondrial; voucher ZSI-HQ-M33049/9; Sreeraj, C.R., Raghunathan, C., Deepa, J. and Karuthapandi, M. MW459189; 450 bp; large subunit ribosomal RNA gene, partial sequence; mitochondrial; voucher ZSI-SbRC-KN3088; Sreeraj, C.R., Raghunathan, C., Deepa, J. and Karuthapandi, M.

Diagnostic characters: Notum raised, elongated and brownish in colour. Large conical papillae all over the notum with larger papillae on the middle of the dorsal part. The hyponotum was light greyish in colour with grey foot with greenish hue. In some individuals, the hyponotum as well as the foot is pale yellowish in colour. The width of the foot is 1/3 in relative width of the hyponotum. The ocular tentacles are exceptionally long in this species with reddish brown in colour and eyespot at the tip. The head is also reddish brown in colour with white speckles (Figure 1a-c).

Distribution: India: Andaman and Nicobar Islands, West Bengal (type locality). *Elsewhere*: Philippines, includingthe Strait of Malacca, Singapore, Thailand, Vietnam, eastern Borneo, and China (Dayrat *et al*., 2019).

Remarks: The animals were found to crawl in mud where it exhibits high level of cryptic behaviour and makes it almost impossible to get noticed. The individuals of the genus Onchidium can be easily identified in the field because of the presence of large, conical, pointed papillae on the dorsal notum of the undisturbed animals. Once disturbed these papillae will be contracted.

Genus ***Platevindex*** Baker, 1938

Platevindex tigrinus (Stoliczka, 1869)

Material examined: M33050/9 (Mollusca Section, ZSI, Kolkata), 3 exs, Length: 3.5±1 cm, 19.12.2018, Coll: Sreeraj CR, Chollangi, Coringa Wildlife Sanctuary, East Godavari, Andhra Pradesh.

Diagnostic characters: Body flattened and notum blackish in colour. Wide, non-pointed and wider papillae found on the notum. Papillae were less in number, not crowded and scattered all over the notum with larger ones with eyespots being on the dorsal mid portion of the notum. Hyponotum dark yellow in colour with reddish brown larger spots scattered all over the hyponotum with density of the spots less towards the foot. Foot black in colour. The anal opening is posterior to the foot in the left side. The ocular tentacles are light brown at the bottom and reddish towards the tip with eyespot at the tip. The head is light brown in colour (Figure 1d-f).

Distribution: India: Andaman and Nicobar Islands, West Bengal (type locality). *Elsewhere*: No reports outside India.

Remarks: The animals were found on the mangrove trunks and on dry (or less moist) mangrove mud. Very rarely found in the moist muddy area. The notum of the individuals seen in the mud were brownish in colour whereas those seen in the trees were mostly blackish.

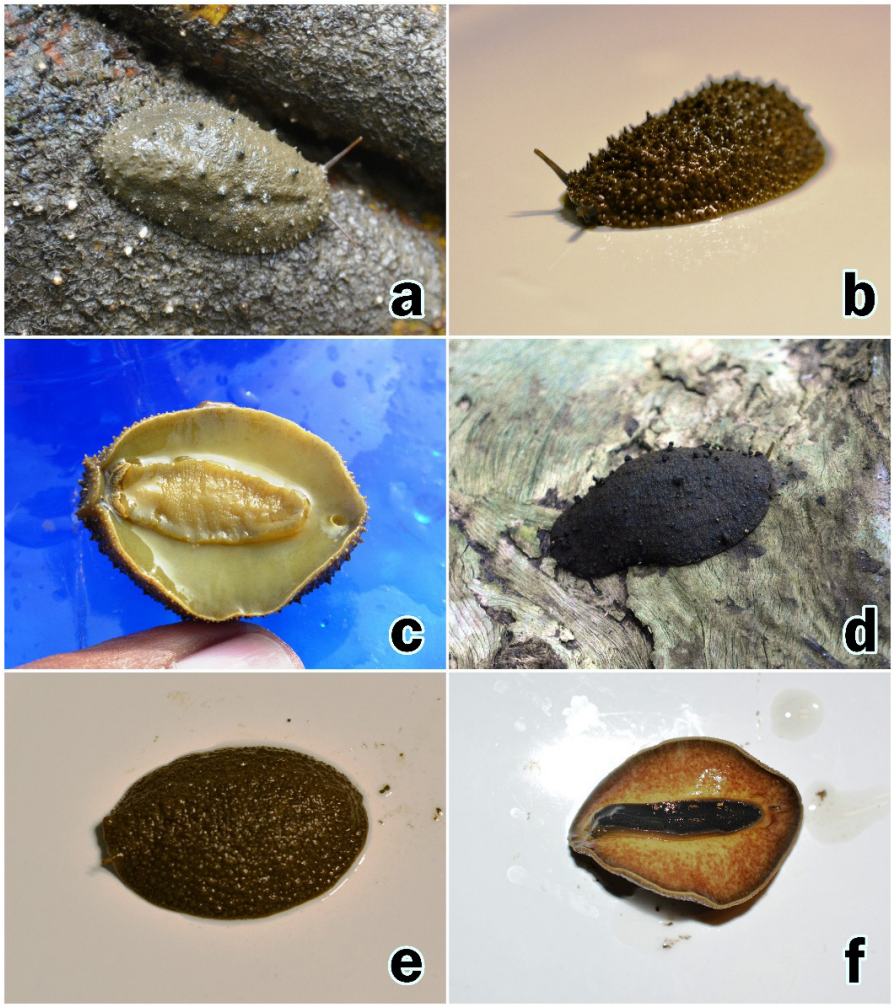

Figure 1. *Onchidium typhae* Buchannan, 1800; **a)** crawling animal in the mud, **b)** animal in the lab showing the long tentacles, **c)** the hyponotum and foot. *Platevindex tigrinus* (Stoliczka, 1869); **d)** animal on the trunk of the large *Avicennia* plant, **e)** animal in lab showing the papillae on the notum, **f)** hyponotum and foot.

Discussion

Onchidium typhae Buchannan (1800) is the type species of the family Onchidiidae by monotypy. This species was originally described from the backwaters of the West Bengal (from Kolkata) and was named for its association with elephant grass (*Typha elephantina* Roxb.). But regrettably, even after 200 years of its description, onchidiid slug remains as the least studied but widely distributed mollusca family in India. Partly this could be attributed to the difficulty in differentiating the species in field and lack of taxonomic expertise in this field. Recently a detailed study on the Onchidiid slugs were carried out and as a result of that many genera as well as species were newly described. Of these, many species such as *Melayonchis aileenae* Dayrat *et al.*, 2017; *Melayonchis eloisae* Dayrat *et al.,* 2017; *Onchidium melakense* Dayrat *et al.*, 2019 and *Paromoionchis penangensis* Dayrat *et al.*, (2019) were described with type material from India also (Mainly form Andaman and Nicobar Islands. Among all the reported species from India, *Peronia verruculata* (Cuvier, 1830) is the most widely distributed species in India (Dayrat *et al.*, 2020).

Presence of onchidiid slugs from the Godavari region, especially from Coringa Wildlife Sanctuary has been reported by many authors (Monolisha & Edward, 2015; Devi *et al.,* 2017; Satyanarayana & Krishna, 2017) but others have ignored this group (eg: Kumar, 2016). The identification of *O.tigrinum* (Monolisha & Edward, 2015) and *O.verruculatum* (Devi *et al.,* 2017) are doubtful as the provided images of the published article are complete mismatch. The present study adds two Mollusca to the faunal diversity of Coringa Wildlife Sanctuary and the Andhra Pradesh. Further, a detailed study will result in finding more onchidiid slugs from the mangrove ecosystem of Coringa Wildlife Sanctuary.

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